

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS Uchida *et al.*

FOR: ENRICHED CENTRAL NERVOUS SYSTEM STEM CELL
AND PROGENITOR CELL POPULATIONS, AND METHODS
FOR IDENTIFYING, ISOLATING, AND ENRICHING FOR
SUCH POPULATIONS

Box Patent Application

Assistant Commissioner for Patents

Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination of the above-identified patent application, please amend the application as set forth below and consider the following remarks.

In the Claims:

Please cancel claims 1-22, 24-26, and 40-43, without prejudice.

Replace the pending claims with the following:

23. (Amended) A population of neurosphere initiating stem cells (NS-IC) produced by:
- (a) combining a population comprising neural cells or neural-derived cells containing a fraction of NS-ICs with a reagent that recognizes a determinant on a cell surface marker recognized by monoclonal antibody AC133 or by monoclonal antibody 5E12;
 - (b) selecting AC133⁺ or 5E12⁺ cells, wherein the selected AC133⁺ or 5E12⁺ cells are enriched in the fraction of NS-ICs as compared with the population of neural cells;
 - (c) introducing at least one AC133⁺ or 5E12⁺ cell to a culture medium capable of supporting the growth of NS-IC; and
 - (d) proliferating the AC133⁺ or 5E12⁺ cell in the culture medium.

27. An *in vitro* cell culture composition comprising:
- (a) a population enriched in AC133⁺ CD45⁻ cells or 5E12⁺ CD45⁻ cells; and
 - (b) a medium capable of supporting the growth the cells.
28. An *in vitro* cell culture composition comprising:
- (a) a population enriched in AC133⁺ CD45⁻ CD34⁻ cells or 5E12⁺ CD45⁻ CD34⁻ cells; and
 - (b) a medium capable of supporting the growth the cells.
29. An *in vitro* cell culture composition comprising:
- (a) a population enriched in AC133⁺ 8G1^{-/lo} cells or 5E12⁺ 8G1^{-/lo} cells; and
 - (b) a medium capable of supporting the growth the cells.
30. An *in vitro* cell culture composition comprising:
- (a) a population enriched in AC133⁺ 8G1^{hi} cells or 5E12⁺ 8G1^{hi} cells; and
 - (b) a medium capable of supporting the growth the cells.
31. An *in vitro* cell culture composition comprising:
- (a) a population comprising at least 50% AC133⁺ or 5E12⁺ neurosphere initiating cells (NS-IC) which stain positive for nestin and, in the presence of differentiation-inducing conditions, produce progeny cells that differentiate into neurons, astrocytes, and oligodendrocytes; and
 - (b) a medium capable of supporting the growth of NS-IC.
32. The composition of claim 31, further comprising a solid support to which the NS-IC are attached.
33. The composition of claim 31, wherein the population of cells has at least 70% AC133⁺ or 5E12⁺ cells.

34. The composition of claim 31, wherein the population of cells has at least 90% AC133⁺ or 5E12⁺ cells.
35. The composition of claim 31, wherein the population of AC133⁺ or 5E12⁺ cells is a substantially pure population.
36. The composition of claim 31, wherein the medium comprises a serum-free medium containing one or more predetermined growth factors effective for inducing multipotent neural stem cell proliferation.
37. The composition of claim 31, wherein the medium further contains a growth factor selected from the group consisting of leukocyte inhibitory factor (LIF), epidermal growth factor (EGF), basic fibroblast growth factor (FGF-2), and combinations thereof.
38. The composition of claim 31, wherein the medium further comprises a neural survival factor.
39. The composition of claim 31, wherein the NS-IC are human.

APPLICANTS: Uchida, *et al.*

REMARKS

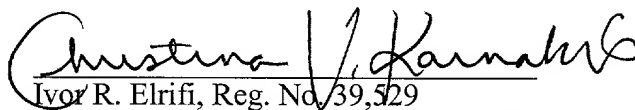
Applicants have amended claim dependent 23 to make it independent by incorporating the limitations of independent claim 22. Applications have also canceled claims 1-22, 24-26, and 40-43, without prejudice or disclaimer. No new matter has been added.

CONCLUSION

This application is believed to be in condition for allowance, and a statement to this effect is respectfully requested. Should any questions or issues arise concerning this application, the Examiner is encouraged to contact the undersigned at (617) 542-6000.

Respectfully submitted,

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Versions with Markings to Show Changes Made

1.-22. (Cancelled)

23. (Amended) [A population of cells produced by the method of claim 22.] A population of neurosphere initiating stem cells (NS-IC) produced by:
- (a) combining a population comprising neural cells or neural-derived cells containing a fraction of NS-ICs with a reagent that recognizes a determinant on a cell surface marker recognized by monoclonal antibody AC133 or by monoclonal antibody 5E12;
 - (b) selecting AC133⁺ or 5E12⁺ cells, wherein the selected AC133⁺ or 5E12⁺ cells are enriched in the fraction of NS-ICs as compared with the population of neural cells;
 - (c) introducing at least one AC133⁺ or 5E12⁺ cell to a culture medium capable of supporting the growth of NS-IC; and
 - (d) proliferating the AC133⁺ or 5E12⁺ cell in the culture medium.

24.-26. (Cancelled)

40.-43. (Cancelled)

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